Developmental Cell
Previews

Stopped in Translation: EMT Control Meets Eukaryotic Elongation

Ying E. Zhang[1,*]
[1]Laboratory of Cellular and Molecular Biology, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892, USA
*Correspondence: zhangyin@mail.nih.gov
DOI 10.1016/j.devcel.2011.02.012

Recently reporting in *Molecular Cell*, Hussey et al. (2011) find that hnRNP E1 inhibits the translation of several genes essential for the epithelial-to-mesenchymal transition (EMT) by blocking eEF1A1 release during translation elongation. Phosphorylation of hnRNP E1 in response to TGF-β signaling disrupts the hnRNP E1-eEF1A1 interaction, triggering EMT.

Epithelial-to-mesenchymal transition (EMT), a process in which epithelial cells are converted into mesenchymal cells before migration, was recognized as a widespread developmental feature in the early 1980s (Greenburg and Hay, 1982). Since then, it has become clear that EMT also plays vital roles in both physiological responses to injuries in the adult and in the onset of pathological conditions, such as organ fibrosis and, more importantly, metastasis of cancers (Thiery et al., 2009). During EMT, epithelial cells lose their apical to basal polarity, characteristic cobblestone shape, and tight cell-cell contacts while gaining mesenchymal markers such as vimentin and fibronectin. Because a diverse range of epithelial and tumor cells undergo EMT as part of the process in reaching their destined fate, a plethora of morphogens, growth factors, hormones, and even nicotine and UV light have been identified as inducers of EMT. These extracellular cues employ a broad range of intracellular mediators to execute EMT through different mechanisms. Recent studies from Philip H. Howe's laboratory have added another layer of complexity by showing that, at least in the context of TGF-β-induced EMT, some of the essential EMT genes are poised to be activated at the step of translational elongation by phosphorylation of a single heterogeneous nuclear ribonucleoprotein E1 (hnRNP E1) (Chaudhury et al., 2010). This group now builds on these findings in a new study (Hussey et al., 2011) published in a recent issue of *Molecular Cell* to provide a detailed account of how hnRNP E1 mediates the translational control of EMT by forming a complex with the eukaryotic elongation factor eEF1A1 and blocking its release from the ribosome A site.

TGF-β induces EMT through the actions of both Smad-dependent and independent mechanisms that activate a large number of transcription factors, including those in the Snail, ZEB, and bHLH families (Xu et al., 2009). For years, this regulation was considered strictly transcriptional until the discovery of an interleukin-like EMT inducer, ILEI, in a polysome-bound mRNA profiling study for metastasis genes (Waerner et al., 2006). A subsequent examination of another critical mediator of TGF-β-induced EMT, Disabled-2 (Dab2), found that its expression was also translationally controlled (Chaudhury et al., 2010; Prunier and Howe, 2005). These studies demonstrated that although the ILEI and Dab2 messages were expressed in abundance in untreated mammary gland epithelial NMuMG and EpH4 cells, they were not loaded onto polysomes for active translation unless the cells were activated by TGF-β. In a reconstituted in vitro translation system, the Howe group identified a 33-nucleotide sequence from the Dab2 3′ untranslated region (UTR) that confers the transcript-specific translational control. This sequence, termed BAT for TGF-β *a*ctivated *t*ranslational element, was predicted to form a stem-loop structure with an asymmetrical bulge. Interestingly, a segment of the ILEI 3′UTR can be folded into a similar stem-loop structure, although its primary sequence was completely different from that of Dab2-BAT. What is more significant is that both Dab2-BAT and ILEI-BAT bind specifically to hnRNP E1, suggesting that the recognition feature of BAT is really the stem-loop structure rather than the RNA sequence.

In this new study, Hussey et al. (2011) identify eukaryotic elongation factor-1 A1 (eEF1A1) as a component of the hnRNP E1-BAT mRNP complex. Binding studies indicated that although both hnRNP E1 and eEF1A1 can bind the BAT element independently, they interact with each other and the presence of the BAT element stabilizes this interaction. During protein translation, the function of eEF1A1 is to facilitate the binding of charged aminoacyl-tRNAs to the ribosome A site. GTP-hydrolysis due to the intrinsic GTPase activity of eEF1A1 causes its release from the ribosome and translocation of aminoacyl-tRNA onto the peptidyl moiety of the growing peptide chain to complete one round of peptide synthesis. Through detailed domain interaction analysis, Hussey and colleagues (2011) showed that hnRNP E1 could bind two of the three eEF1A1 domains, potentially locking eEF1A1 in a conformation that is lodged in the ribosome A site. To test this hypothesis, the authors assembled translation complexes on a poly(U)$_{37}$-BAT RNA template in the presence or absence of hnRNP E1, and annealed a labeled oligodeoxynucleotide probe to this template for monitoring the passage of ribosomes, whose intrinsic helicase activity would displace the labeled probe. In the absence of hnRNP E1, the probe segregated through sucrose sedimentation gradient with the free RNA fraction, but in the presence of hnRNP E1, this probe cosedimented with the 80S ribosomes, indicating that the translation apparatus was stalled at the elongation step. Further experiments showed that in the presence of hnRNP E1, eEF1A1 became permanently associated with the ribosomes in the GDP-bound form. Thus, the BAT element essentially functions as an affinity trap in

the 3′UTR, forcing hnRNP E1 to complex with eEF1A1 and inhibit translation elongation.

The above analyses sketched a static model of translational control (Figure 1) in which hnRNP E1 blocks translation by preventing the release of eEF1A1 from ribosomes, but how is this mechanism regulated by TGF-β signaling? Clues from the ability of alkaline phosphatase and PI3K inhibitor LY294002 to confer translational silencing activity to the TGF-β-treated extracts suggested that TGF-β might regulate the translational control by phosphorylation. Indeed, inspection of the hnRNP E1 sequence identified an Akt consensus phosphorylation site at serine 43 (S43), and subsequent experiments confirmed that phosphorylated hnRNP E1 was unable to bind either BAT or eEF1A1.

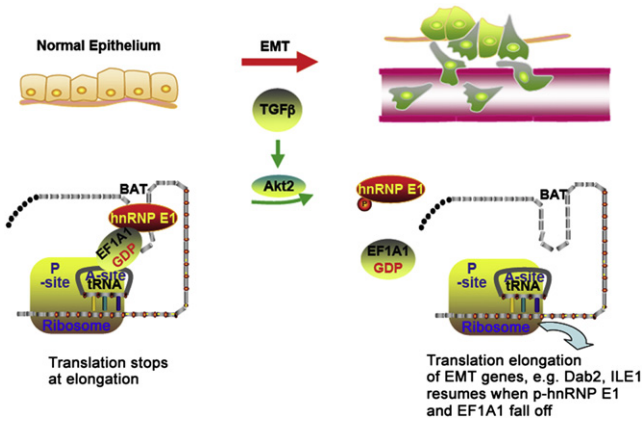

Figure 1. A Model for a BAT-Mediated Regulatory Mechanism of Translational Control

In epithelial cells, the BAT element in the 3′UTR of EMT genes (e.g., Dab2, ILE1) traps hnRNP E1 and eEF1A1 in a complex at the ribosome A site, blocking the conformational change induced by GTP-hydrolysis in eEF1A1. This forces ribosomes to stall on the mRNA template with GDP-eEF1A1 and blocks translation elongation. During EMT, TGF-β signaling induces phosphorylation of hnRNP E1 via Akt2 and disrupts the hnRNP E1 and eEF1A1 complex, thereby allowing eEF1A-GDP release from the ribosome and, subsequently, translocation of aminoacyl-tRNA to the petide moiety of the growing peptide chain, which restores the translation of EMT genes.

Does phosphorylation of hnRNP E1 alone account for the full activation of genes essential for EMT by TGF-β in vivo? To address this issue, the authors generated a stable hnRNP E1-knockdown cell line in which ILEI mRNA was abundantly associated with polysomes irrespective of TGF-β treatment. The cytosolic extract isolated from these cells failed to inhibit translation of a reporter BAT template in the cell-free in vitro translation reaction. Reintroducing wild-type hnRNP E1, but not the S43A mutant that cannot be phosphorylated, restored the translation inhibitory activity of the cytosolic extract and returned the ability for TGF-β to induce ILEI polysome-association. Finally, to address if modulating hnRNP E1 has a direct impact on metastasis, Hussey et al. (2011) generated a stable knockdown line of the tumorigenic but noninvasive MCF7 cells. When injected into nude mice through tail veins, these knockdown cells readily metastasized into the lungs.

Given the remarkable morphological and functional differences between epithelial and mesenchymal cells and the lethal consequence of metastasis, one cannot help wondering why nature has assembled the entire cellular machinery for EMT ready to be dispatched by a simple phosphorylation switch. One way to think about this may lie in the fact that EMT is really a prelude to cell migration while leaving untouched the developmental fate or functional identity of the affected cells. This is certainly true for cancers as metastasis does not alter the oncogenic nature of tumor cells. As scientific facts often run counter to intuitive perception, perhaps a low genetic barrier and the right environmental cue are just what is needed to set the cells moving.

REFERENCES

Chaudhury, A., Hussey, G.S., Ray, P.S., Jin, G., Fox, P.L., and Howe, P.H. (2010). Nat. Cell Biol. *12*, 286–293.

Greenburg, G., and Hay, E.D. (1982). J. Cell Biol. *95*, 333–339.

Hussey, G.S., Chaudhury, A., Dawson, A.E., Lindner, D.J., Knudsen, C.R., Wilce, M.C., Merrick, W.C., and Howe, P.H. (2011). Mol. Cell *41*, 419–431.

Prunier, C., and Howe, P.H. (2005). J. Biol. Chem. *280*, 17540–17548.

Thiery, J.P., Acloque, H., Huang, R.Y., and Nieto, M.A. (2009). Cell *139*, 871–890.

Waerner, T., Alacakaptan, M., Tamir, I., Oberauer, R., Gal, A., Brabletz, T., Schreiber, M., Jechlinger, M., and Beug, H. (2006). Cancer Cell *10*, 227–239.

Xu, J., Lamouille, S., and Derynck, R. (2009). Cell Res. *19*, 156–172.